

CLONAL PROPAGATION OF ARANDA, ASCOCENDA, CATTLEYA BY LEAF TISSUE CULTURE

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INTRODUCTION

Monopodial orchids seem to differ from sympodial orchids in their response to tissue culture. We chose both monopodial orchids and sympodial orchids as material to study their proliferation ability by leaf tissue culture. Originally leaves of aseptic mericlone plantlets of Aranda Noorah Alsagoff (monopodial), Cattleya bowringiana x C. forbesii (sympodial) and Den. Alice Spalding (sympodial) were used. Subsequently, leaves of mature nursery plants of Aranda Wendy Scott, Aranda Christine No. 27, No. 130, Ascocenda Hilo Rose x Vanda Josephine (monopodial) and Den. Sunny (sympodial) were experimented with. Numerous plantlets from all, except the Dendrobiums, were successfully obtained by leaf tissue culture.

MATERIALS AND METHODS

Leaves from Aranda Noorah Alsagoff and Cattleya bowringiana x C. forbesii mericlones were cut in the middle into two parts, so that one part contained the leaf tip and the other part contained the leaf base. Both parts of the leaf were cultured separately in test tubes of 3 different types of liquid media A, B, and C (Table I). All cultures were placed under continuous illumination with Sylvania Glo-Lux 30 w tube and agitated with back and forth movements of a shaker 16 hours a day at a temperature of 28°C.

Whole young leaves between 1 to 3 cm long and 0.5 to 1 cm wide were obtained from mature nursery plants of Aranda Christine No. 27 and No. 130, Aranda Wendy Scott, Ascocenda Hilo Rose x Vanda Josephine and Den. Sunny. The leaves were sterilized in a 10% chlorox solution for 15 to 20 minutes and suspended in medium C. Only Aranda Christine 130 was suspended in media A, B and C. The cultures were placed under the same condition as the above mentioned mericlone young leaves.

TABLE I
MEDIA FOR LEAF TISSUE CULTURE

Medium A	#	Murashige-Skoog inorganic salts	full strength
		Thiamine	1.0 mg/l
		2-4-D	1.0 mg/l
		6-amino-benzyladenine	0.5 mg/l
		Sucrose	30.0 mg/l
		pH 5.0	

	#		
Medium B		Vacin and Went inorganic salts	full strength
		Coconut water	500 ml/l
		Sucrose	20 g/l
		pH 5.0	
Medium C		Murashige-Skoog inorganic salts	full strength
		Thiamine	1.0 mg/l
		myo-Inositol	100.0 mg/l
		Glycine	1.0 mg/l
		Nicotinic acid	0.25 mg/l
		Pyridoxin HCl	0.25 mg/l
		2-4-D	2.0 mg/l
		6-amino-benzyladenine	2.0 mg/l
		Coconut water	150.0 ml/l
		Sucrose	30.0 g/l
		pH 5.0	

RESULTS AND DISCUSSIONS

The results of leaf tissue culture are shown in Table II.

TABLE II
RATE OF PROLIFERATION OF LEAF TISSUE CULTURE
IN MEDIA A, B AND C

Leaf from Mericlone Plantlet:	Medium A	Medium B	Medium C
Aranda Noorah Alsagoff (monopodial)			
Leaf tip	—	—	—
Leaf base	—	++	+++
Cattleya bowringiana x C. forbesii (sympodial)			
Leaf tip	—	—	—
Leaf base	++	+	+++
Den. Alice Spalding (sympodial)			
Leaf tip	—	—	—
Leaf base	—	+	—
Leaf from Mature Nursery Plants:			
Aranda Wendy Scott (monopodial)			
Whole Leaf			+++
Aranda Christine No. 27 (monopodial)			
Whole Leaf			+++
Aranda Christine No. 130 (monopodial)			
Whole Leaf	—	++	+++
Ascocenda Hilo Rose x Vanda Josephine			
Whole Leaf (monopodial)			+++
Den. Sunny (sympodial)			
Whole Leaf			—

No proliferation —
1 plantlet formed +

Good proliferation ++
Excellent proliferation +++

Both media A and B are modified from Churchill et. al. *Orchid Dig* 34: 271-273 and *Amer. Orchid Dig.* 34: 100-113.

All leaf tips did not proliferate after culturing for a period of 9 weeks. Leaf tips in medium B were still alive but none of them proliferated. Leaf tips in medium A and C, turned brown and died. Leaf bases of *Aranda Noorah Alsagoff* proliferated in both media B and C (plate I). Leaf bases of *C. bowringiana* x *C. forbesii* proliferated in media A and C (plate II) but formed only one plantlet in medium B. Nine duplicates were made of all the above cultures and all showed consistent results. The whole leaves of mature plants of *Aranda Christine* No. 27, and No. 130 (plate III) *Aranda Wendy Scott* (plate IV) and *Ascocenda Hilo Rose* x *Vanda Josephine* all formed excellent proliferations in medium C. *Den Alice Spalding* (mericlone plantlet) and *Den. Sunny* (mature plant) all failed to proliferate. They did produce some callus-like tissue in solid medium C, but differentiated plantlets except for some leaf bases of *Den. Alice Spalding* which formed single plantlets in medium B.

All leaves which we were experimented with survived in medium B although they might or might not have produced proliferate bodies. Medium B contains quite a high concentration of coconut water (50%). There must be a compound in the coconut water which can prevent, in leaves in-vitro cultures, senescence and gradual death after excision from the mother plants. Zeatin, a kind of natural cytokinin in coconut water may take part in this role. 6-amino-benzyladenine in medium A does not seem to be as effective as coconut water in preventing excised leaves from senescing.

Excised monopodial orchid leaves turned brown and died after culturing in medium A, but survived and proliferated in both medium B and medium C which contain coconut water. Monopodial leaves may contain quite a high level of endoauxin, so that they easily senesce. and an extra amount of cytokinin is required to counteract this endoauxin reaction. This also could explain why proliferating bodies are formed in medium B which has no extra auxin added in the medium. In contrast to the above, *Cattleya* leaves may contain a lower level of endoauxin. 6-amino-benzyladenine in medium A is enough to prevent them from senescence. However auxin in coconut water alone is not enough to stimulate the formation of protocorms and additional auxin in the medium is required for the formation of proliferating bodies.

Medium C contains both coconut water and additional plant hormones. Thus both the monopodial orchids and *Cattleya* form proliferating bodies.

Since all the media, A, B, and C contain quite a high amount of plant hormones, or coconut water, the protocorm-like bodies were transferred to a regular liquid mericlone medium, namely Vacin and Went inorganic salt, 15% coconut water and no sugar, as soon as they formed, in order to minimise the chances of producing variants. These leaf protocorms are kept in this medium to serve as stocks.

When transferred to a solid medium the protocorm-like bodies all differentiated into plantlets. Numerous plantlets were produced from these leaf protocorms in a period of 9 months. They all have a normal appearance and many of them have already been transplanted into community pots in the nursery.

In our experiment, all leaf portions containing the leaf base produced proliferating bodies in medium C. We believe that the leaf bases have some young cells which may or may not have undergone cell division but are not fully expanded cells and hence are easily stimulated to become meristematic tissue. The leaf tip cells which we have used may have already matured to a certain extent so that

they are difficult to stimulate to become meristematic tissue. Very young leaf tips, such as the leaf primordia which we removed from a swollen (top or axillary) bud in our shoot tip culture did produce proliferate bodies in medium C.

We believe this success in leaf base portions is not because they have some of the meristematic tissue of the axillary bud attached to it, as surface sterilization with Clorox solution bleached the excised leaf base margins to a depth 0.1 to 0.2 cm and any attached meristematic tissue must have been killed before the culture procedure commenced.

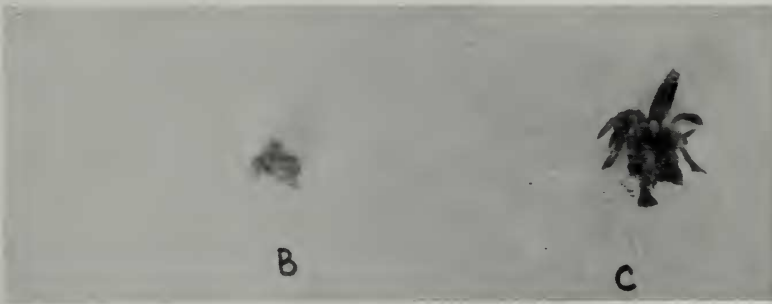


Plate 1. Leaf bases of Aranda Noorah Alsagoff proliferated in both media B and C (x 0.5).



Plate 2. Leaf bases of Cattleya bowringiana x Cattleya forbesii proliferated in media A and C (x 0.5).



Plate 3. The whole leaves of mature plants of Aranda Christine No. 130 proliferated in both medium B and medium C (0.5).

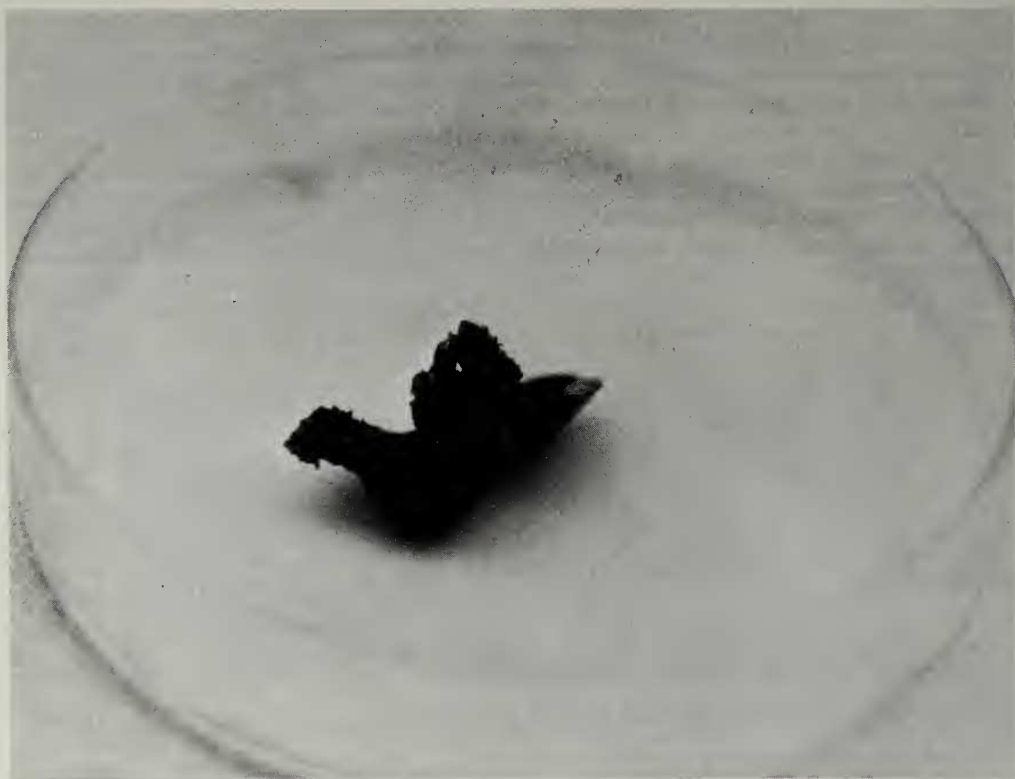


Plate 4. The whole leaves of mature plants of *Aranda Wendy Scott* proliferated in medium C (x 1).



Plate 5. The whole leaves of mature plants of *Ascocenda Hilo Rose* x *Vanda Josephine* proliferated in medium C (x 0.8).

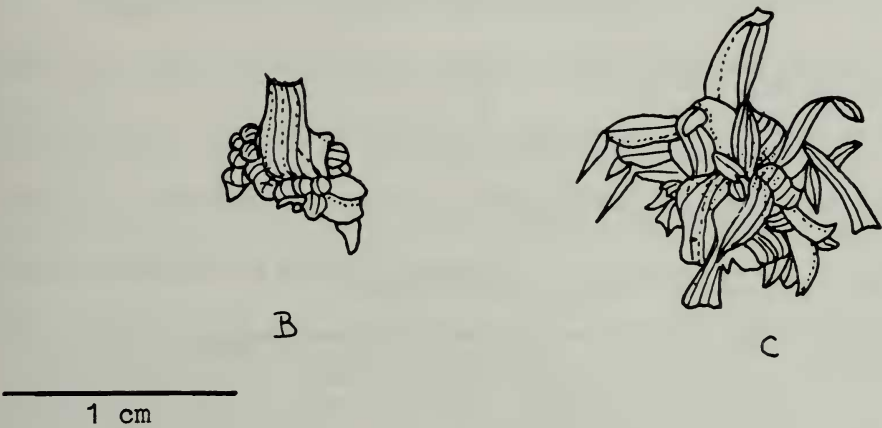


Fig. I. Leaf bases of *Aranda Noorah Alsagoff* proliferated in both media B and C.

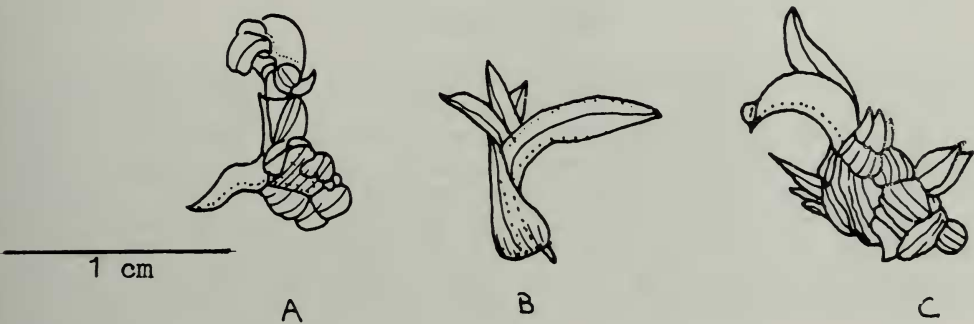


Fig. II. Leaf bases of *Cattleya bowringiana* x *Cattleya forbesii* proliferated in media A and C.

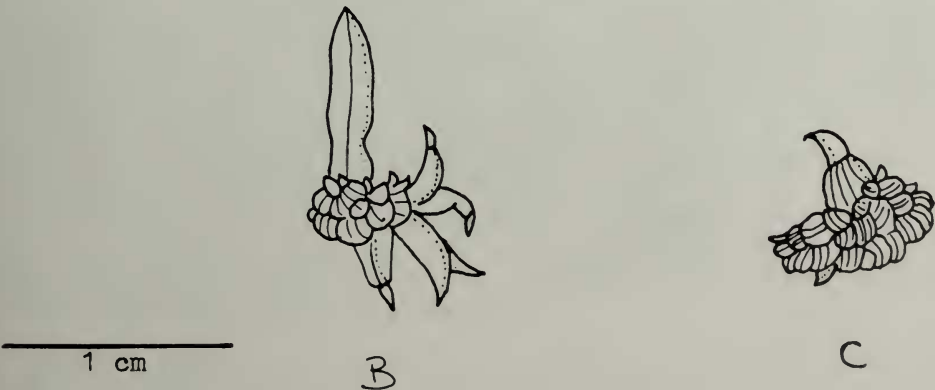


Fig. III. The whole leaves of mature plants *Aranda Christine* No. 130 proliferated in both medium B and medium C.

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